Reference: *Biol. Bull.* **167**: 467–476. (October, 1984)

DIRECT ACCESS OF IONS TO THE SQUID STELLATE GANGLION GIANT SYNAPSE BY AORTIC PERFUSION: EFFECTS OF CALCIUM-FREE MEDIUM, LANTHANUM, AND CADMIUM

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Abstract

The giant synapse in the squid stellate ganglion has served as a model in the understanding of normal synaptic transmission, but has not been used extensively in the study of changes in external ion concentrations or pharmacological agents. This anomaly is due primarily to the substantial diffusion barrier that exists between the synapse and the bathing medium. The present study describes a technique for the rapid introduction of substances into the synapse by perfusion through the arterial blood supply that improves access by at least 50-fold. This is demonstrated by treatments known to block the Ca^{2+} activated release of transmitter from the nerve terminal: Ca^{2+}-free medium, La^{3+}, and Cd^{2+}. Whereas such treatments take 20 minutes to 3 hours to block transmitter release with bath application, with perfusion they act within a few seconds. The excitatory postsynaptic potential (EPSP) is reduced below action potential threshold in 5 to 22 seconds, and disappears completely in less than a minute. In addition, the use of pressurized O_2 to drive the perfusate through the preparation eliminates the need for superfusion with O_2 and aids in the long term maintenance of the ganglion. This study confirms the important role of Ca^{2+} in the release of transmitter at the giant synapse, and opens up this neurobiologically important preparation for ionic and pharmacologic evaluation.

Introduction

The giant synapse in the stellate ganglion of the squid is a unique preparation for the study of chemical transmission in that both the pre- and postsynaptic axons are of sufficient size to allow microelectrode penetration (Bullock and Hagiwara, 1957). This has allowed a detailed analysis of the physiology of synaptic transmission, in particular the ionic currents associated with the release of transmitter from the presynaptic terminal (*e.g.,* Kusano *et al.,* 1967; Miledi, 1973; Llinas *et al.,* 1981; Charlton *et al.,* 1982). However, relatively little is known about the pharmacological properties of the giant synapse. The principle reason for this appears to be the high diffusion barrier that exists between the external bathing solution and the synapse itself, as noted in previous studies (Bryant, 1958; Webb *et al.,* 1966; Kelly and Gage, 1969; Lester, 1970; Erulkar and Weight, 1977). Attempts to evaluate effects of pharmacological agents or ions on the pre- or postsynaptic axons have been limited or unsuccessful. For example, Bryant (1958) explored the action of a number of pharmacological agents applied to the ganglion via the bathing medium. Only the non-

Received 30 May 1984; accepted 25 July 1984.

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specific effect of a gradual decline in the excitatory postsynaptic potential (EPSP) was noted and this occurred over a period of 30 minutes or more. Even experiments examining the effect of changes in external concentrations of mono- and divalent cations have been limited by access to the giant synapse. Thus, the diffusion barrier restricted experiments to one or two changes of K^+ concentrations per experiment in a recent study (Erulkar and Weight, 1977) while in other studies the time taken for equilibration of release following changes in external Ca^{2+} was 20 minutes to 3 hours (Miledi and Slater, 1966; Lester, 1970). Other examples in the literature include: 3-aminopyridine (Llinas *et al.*, 1976) and L-glutamate (Kelly and Gage, 1969) and it is likely that the same difficulty has been encountered with other bath applied substances but that these results have never been reported. The long delay between application of a test substance in the bath to its access to the synapse excludes the examination of the acute effects of the agent, and delayed effects are difficult to interpret since there is an increased likelihood that they are due to a secondary factor.

A method of introducing substances through the arterial blood supply of the ganglion is presented in the present report, reducing the access time to the synapse by up to two orders of magnitude. This is illustrated by the rapid actions of Ca^{2+}-free medium, $LaCl_3$, and $CdCl_2$ in blocking synaptic transmission. All of these treatments are known to inhibit the quantal release of transmitter from the nerve terminal by interfering with the voltage triggered influx of calcium ions that normally activates the release mechanism (reviewed by Hagiwara and Byerly, 1981). The experiments described in this study illustrate the improved access of substances into the ganglion with this technique, demonstrate the compatibility of arterial perfusion with recording, and, in addition, present a novel method of oxygenating experimental preparations. Preliminary results of this study have appeared in abstract form (Stanley and Adelman, 1982).

Materials and Methods

Gross dissection

Small squid of less than 14 cm mantle length were selected for study of the giant synapse, since in larger squid the ganglion is less transparent and more difficult to penetrate with microelectrodes.

The initial dissection was similar to that described by Miledi and Slater (1966). The squid was decapitated and the body was immediately placed on a dissection table with fresh oxygenated sea water continuously passing over it. An incision was made up the ventral midline and the mantle was laid flat to reveal the organs of the body (Fig. 1a). The two stellate ganglia lie on the mantle, on either side of the gut muscular wall. The ink sac, gills, reproductive organs, and syphon muscle were removed, and the muscular tube containing the gut was cut up each side. The digestive organ was removed, care being taken not to damage the blood vessels and the pair of nerves from the head ganglia (stellate ganglion pre-nerves) immediately below it (Fig. 1b). The pre-nerves were tied off with cotton thread at their most rostral ends and dissected free from the underlying musculature, back to the holes in the gut muscle where they descend to innervate the ganglia. The muscle overlying the two ganglia was then carefully removed.

Cannulation of the artery

The single medial artery that supplies both ganglia is located between the gut and its muscular wall and can be recognized by its smooth, thick-walled appearance. A

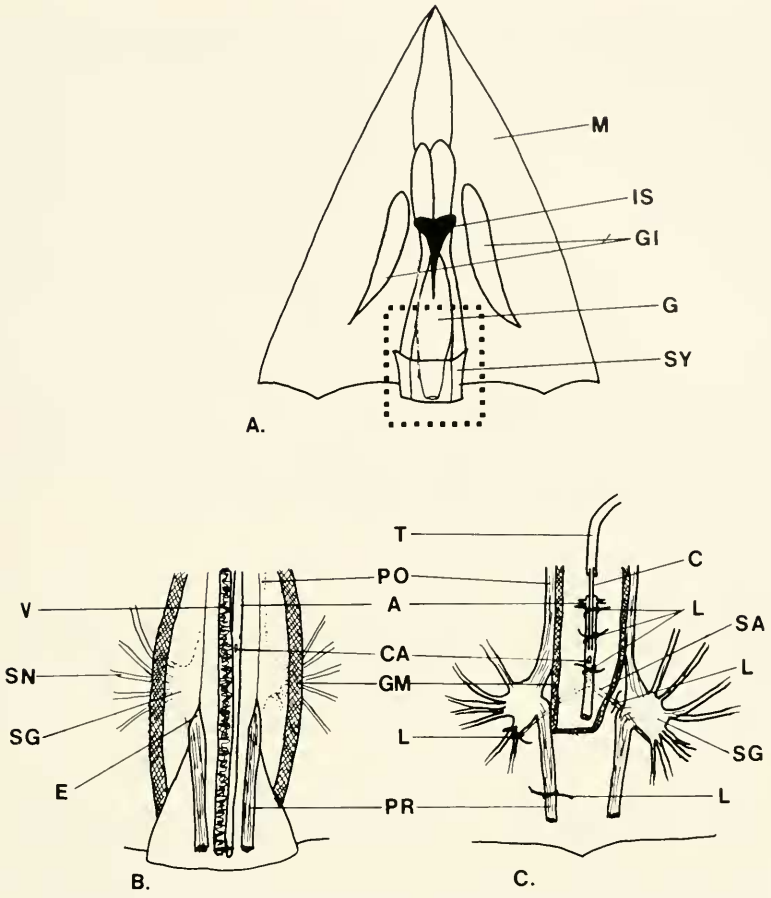

FIGURE 1. Dissection and perfusion technique. A. Gross dissection. The mantle was cut up the midline and reflected to reveal the organs in the mantle cavity. B. Enlarged region denoted by dotted lines in A after removal of the syphon, the ventral gut musculature, and the digestive gland. C. As in B but after removal of the vein, trimming of the remaining gut muscle to reveal the ganglia, and cannulation of the aorta.

M, mantle; IS, ink sack; GI, gill; G, digestive gland; SY, syphon; V, vein; SN, stellar nerve; SG, stellate ganglion; E, point of descent of the prenerve through the digestive gland muscle wall; T, tubing; PO, medial stellar nerve—containing the largest postsynaptic giant axon; CA, point of exit and descent of the common stellate artery from the aorta; GM, cut edge of the digestive gland muscle wall; L, ligature; PR, pre-nerve—containing the presynaptic giant axon; C, cannula; SA, stellate artery.

second, larger and wrinkled vessel is a vein which can be carefully stripped off. The artery supplies both ganglia from a single branch (which we term the common stellate artery) that passes through the underlying muscle and bifurcates to the ganglia (the right and left stellate arteries). After passing the ganglia, the stellate arteries also supply the rostral third of the mantle musculature.

Perfusion of the ganglia requires several ligatures (Fig. 1c). One ganglion, the right, was selected for the present study. The medial aorta was tied off with 6–0 suture just rostral to the common stellate branch. Ligatures were tied loosely at two points on the aorta, caudal to the common stellate artery, in readiness for the cannula. The rostral ligature of this pair was positioned very closely to the common stellate artery

to minimize leakage through small branches that supply the outer gut musculature. The caudal ligature passed through a portion of the underlying muscles. This ligature prevents tension on the cannula from tearing the common stellate artery during the dissection. The medial aorta was then cut approximately 1 cm distal to the common stellar nerve and the cannula—a blunted, 30 g syringe needle connected to PE 10 polyethylene tubing—was inserted and tied in place with its tip directly over the common stellate artery. Two further ligatures were tied: the right stellate artery was tied off proximal to the right ganglion, and the left stellate artery was tied off immediately distal to the left ganglion but proximal to where it bifurcates and descends into the mantle. Other small artery branches may require suturing in individual squid.

The ganglion was dissected free from the mantle by gently lifting the left pre-nerve by means of its ligature, and cutting the membranes that attach it to the mantle. In the process all the nerves emanating from the ganglion were cut except the last (medial) stellar nerve which contains the third order postsynaptic giant axon of interest in this study. This nerve was dissected free for 15 mm and tied off with silk suture. The left ganglion and nerves were then removed, together with a portion of the overlying outer gut muscle between the ganglion and the cannula, and transferred to an experimental chamber, a silastic coated 35-mm petri dish.

Fine dissection

The ganglion was pinned out in the experimental chamber under an atmosphere of O_2 by the threads attached to the pre-nerve and the medial stellar nerve, the remaining piece of gut muscle underlying the cannulated aorta, and by pins through three of the cut stellar nerves.

The stellate ganglion is enveloped by a "capsule" of smooth muscle that continually contracts and relaxes. This capsule must be removed on the upper surface of the ganglion to allow access to the giant synapse. Fortunately, the arteries enter the ganglion from the rear so that cutting a window in the capsule does not affect arterial perfusion of the ganglion. The fin nerve, which passes from the pre-nerve to the medial stellar nerve without entering the ganglion, was left intact except in experiments requiring extracellular recording from the medial stellar nerve. In these experiments a short segment of the fin nerve was removed to allow the recording electrodes to come into close contact with the giant axon. Cutting the fin nerve close to the ganglion was avoided in most cases (but is possible) to avoid damage to, and hence leakage from, the arteries supplying this nerve.

Introducing test substances by changing the perfusion medium

The arterial cannula was connected, via a short length of polyethylene tubing, to a four-way miniature stopcock (Hamilton) that allowed changes in the perfusion medium. Perfusion was carried out by forcing artificial sea water (ASW, composition in m*M:* NaCl, 423.0; KCl, 9.0; $CaCl_2$, 9.3; $MgCl_2$, 22.9; $MgSO_4$, 25.5, Hepes 5.0) through the ganglion with gas pressure from a compressed O_2 tank (12–18 psi). This method of perfusion is of a "constant pressure" type, similar to gravity feed, but allows much greater forces. Constant pressure perfusion was chosen over the alternative of a "constant flow rate" (as with a peristaltic or syringe pump). With the constant pressure method the absolute flow rate is more difficult to control (but was reasonably constant for any preparation) but pressure changes during switching from ASW to test solutions were negligible. This avoided movement artifacts during microelectrode recording. Measured flow rates ranged from 0.1 to 0.5 ml/min, depending in part on leaks through small artery branches that were not ligatured.

An additional major advantage of the perfusion method was the very effective introduction of O_2 into the preparation. Transmission at the giant synapse is known to be highly dependent on an adequate O_2 supply (Bryant, 1958). Since the perfusion solution is forced into the ganglion under a high pressure of O_2, the perfusate is presumably saturated or super-saturated with O_2 when it enters the lower pressure of the ganglion. In fact, with this method it is unnecessary to supply any additional source of O_2 for many hours of study.

The test substance was dissolved in ASW and was then introduced by manually turning the tap on the stopcock. Test solutions were: Ca^{2+} free medium (composition as ASW but $CaCl_2$ 0 m*M*, $MgCl_2$ 65 m*M*); $LaCl_3$ (1 or 3 m*M*); and $CdCl_2$ (1 m*M*). Experiments were carried out at room temperature (22–24°C).

Stimulation and recording techniques

The pre-nerve was stimulated by a square wave pulse of electricity originating from a stimulator (Grass S48) via a stimulus isolation unit (Grass SIU 5) and a pair of silver wire electrodes hooked under the nerve.

Extracellular action potentials in the stellar nerve were recorded by means of a pair of silver wire electrodes connected to a differential amplifier (WPI, DAM6) and a storage oscilloscope (Tektronix D11).

Intracellular recording was carried out by conventional microelectrode techniques. A 3 *M* KCl filled micropipette (5–15 Mohms) was connected via a holder and a probe to a DC amplifier with high input impedance and unit gain (WPI S-7000 system). The output of the amplifier was displayed on the storage oscilloscope (Tektronix D11) to record action potentials and EPSPs, while resting potentials were displayed on the digital meter of the S-7000.

Results

In initial experiments access of perfusate into the ganglion was examined with ASW containing the dye carmine red. Perfusion of dye resulted in a rapid color change of the whole preparation and revealed an interdigitating network of vessels within the ganglion. Dye was detected throughout the region of the ganglion containing nerve fibers and cell bodies and also the first few mm of the pre-nerve and the stellar nerves. A large vessel was usually observed passing very closely to the giant synapse. Fixation of the ganglion by perfusion was also very rapid and resulted in excellent maintenance of morphology (Martin and Miledi, 1975; D. W. Pumplin and E. F. Stanley, unpub. obs.).

In order to examine the access of substances to the giant synapse, it is necessary to select a treatment that is specific for synaptic transmission. This was achieved by blocking transmitter release by two cations known to interfere with calcium flux, La^{3+} or Cd^{2+}, or by Ca^{2+}-free medium.

Effect of La^{3+}. The substantial diffusion barrier to the giant synapse was confirmed by adding $LaCl_3$ (3 m*M*) to the bathing medium of a ganglion incubated without perfusion but with the capsule removed and O_2 continually passing over the preparation. Extracellular stimulation and recording techniques were used to examine transmission across the synapse stimulating the pre-nerve while the postsynaptic giant axon action potential was recorded from the medial stellar nerve. In normal ASW the synapse transmits the impulses for many hours at a stimulus frequency of 1 Hz. After addition of La^{3+}, transmission continued normally for 30 minutes but by 36 minutes the synapse would not transmit 10 consecutive stimuli at 1 Hz, and by 39 minutes there was complete failure.

The effect of perfusing $LaCl_3$ on transmission was compared with that of bath application by use of intracellular recording techniques. The postsynaptic giant axon was penetrated by a microelectrode and a 60 to 70 mV resting potential was recorded. The pre-nerve was stimulated at 1 Hz evoking an EPSP/action potential complex in the postsynaptic giant axon. This preparation was highly stable. The resting potential and synaptic transmission were maintained for several hours of study. La^{3+} (1 m*M*) perfusion resulted in a small decline in the resting potential, failure of the action potential component of the EPSP/action potential complex and a gradual reduction of the EPSP to below the detectable level (Fig. 2). The effect of La^{3+} on the EPSP was irreversible, even after washout periods of 20 minutes, although the RMP did recover. Experiments examining the washout of ions from the preparation were therefore carried out using another ion that blocks the entry of Ca^{2+} into the terminal, Cd^{2+}.

Effect of Cd^{2+}. $CdCl_2$ had a similar effect of $LaCl_3$. The EPSP declined below threshold for an action potential in 7 ± (S.D.) 1 s (n = 4) and to below the level of detection in 14 ± 4 s (Figs. 3a, 4), (minimum 6 s and 12 s, respectively). A decline in the resting potential (10 to 20 mV) was also noted (Fig. 4a), but Cd^{2+} did not block antidromically evoked potentials in the giant axon. Unlike La^{3+}, the effect of Cd^{2+} on synaptic transmission was reversible. Switching the perfusion solution back to ASW resulted in the gradual recovery of the resting potential, and in the reappearance of the EPSP to threshold for an action potential in the postsynaptic axon (Fig. 4b).

Effect of Ca^{2+}-free ASW. The effect of Ca^{2+}-free sea water on synaptic transmission was also examined by intracellular recording from the postsynaptic giant axon. The effect of removing Ca^{2+} was similar to that of the action of La^{3+} or Cd^{2+}, a gradual

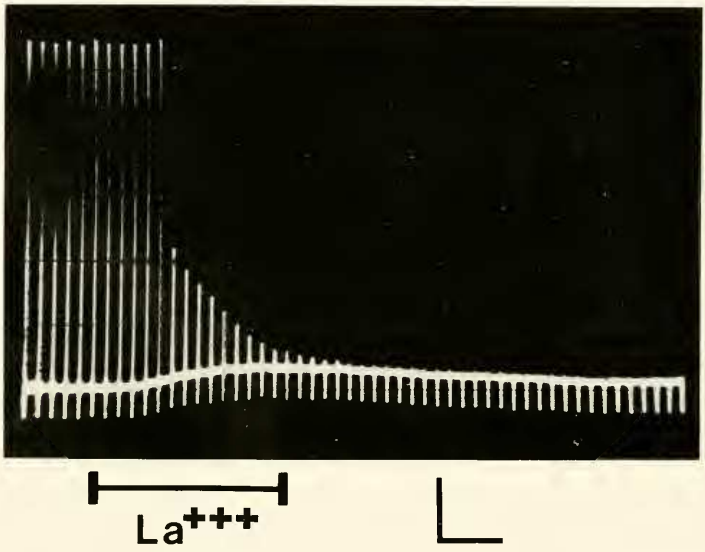

FIGURE 2. Effect of La^{3+} (1 m*M*) infusion (horizontal bar) on potentials recorded intracellularly from the postsynaptic giant axon (stimulus frequency 1 Hz). La^{3+} resulted in a disappearance of the synaptically evoked action potential followed by a decline in the EPSP and a coincident decline in the resting potential. Re-infusing plain ASW (onset at the end of the horizontal bar) resulted in a gradual recovery of the resting potential, but the EPSP did not reappear. The down-going trace deflections are due to a stimulus artifact and obscure the negative after potential of the action potential. Calibration: amplitude = 10 mV, time = 5 s.

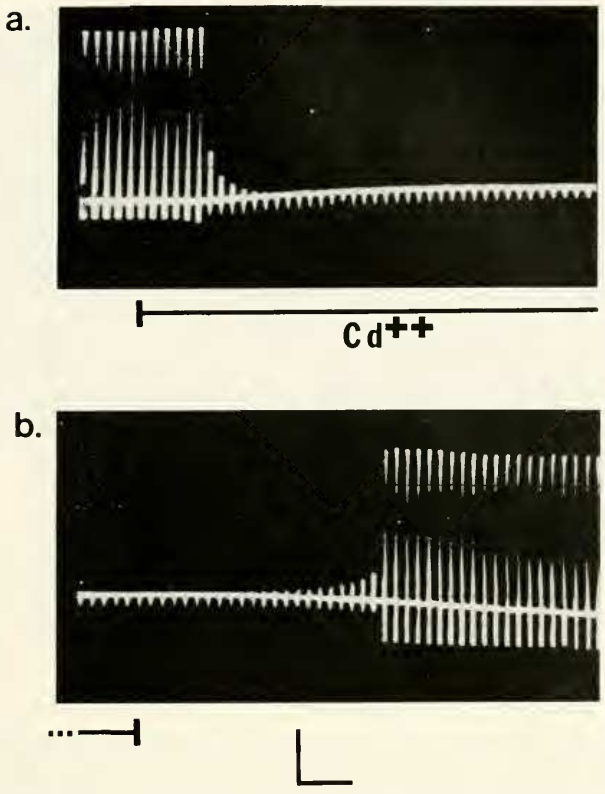

FIGURE 3. Effect of Cd^{2+} on potentials recorded from the postsynaptic giant axon. a. Infusion of Cd^{2+} (1 m*M*) resulted in the disappearance of the synaptically evoked action potential, elimination of the EPSP, and a decline in the resting potential. b. Washout of Cd^{2+} was followed by a reappearance of the EPSP and the action potential and a gradual recovery of the resting potential (retouched). Calibration: amplitude = 20 mV, time = 5 s.

reduction in the EPSP to below threshold and eventually to a complete disappearance (Fig. 5). However, Ca^{2+}-free medium reduced the EPSP at a slower rate than the other treatments, eliminating the synaptically evoked action potential in 13 ± 7 s, and the EPSP in 35 ± 12 s (n = 4) (minimum 5 s and 18 s, respectively). Reperfusion with Ca^{2+}-containing ASW resulted in transmission recovery.

DISCUSSION

The blood system of the squid has been described by Williams (1909), but the arterial system supplying the stellate ganglia was not discussed in detail in this classical study. The heart is situated between the gills in the caudal region of the mantle cavity and the aorta runs rostrally, penetrates the muscular coat of the digestive gland, and passes between the digestive gland muscle wall and the gland itself, terminating in the head. Just rostral to the level of the stellate ganglia the aorta sends a single branch through the digestive gland muscle wall which bifurcates to supply the two stellate ganglia. These arteries continue beyond the ganglia as the major blood supply for the rostral mantle. Thus, cannulation of the aorta together with appropriately positioned

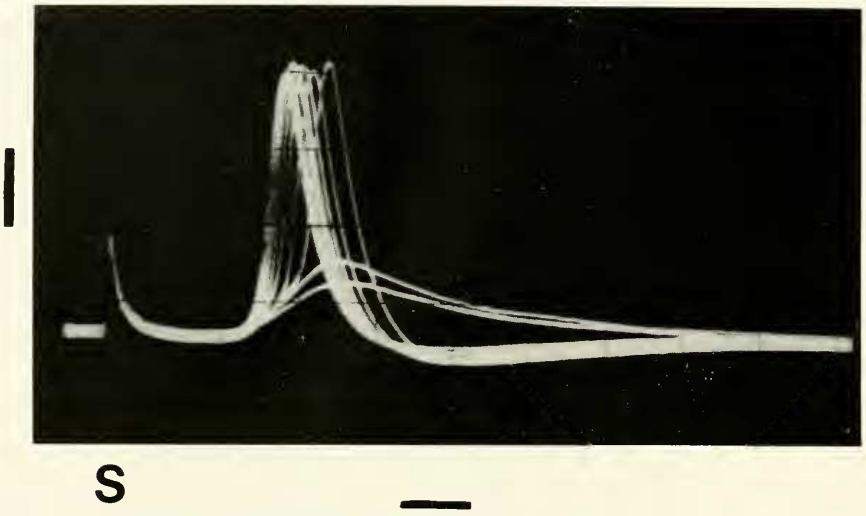

FIGURE 4. Effect of Cd^{2+} infusion on intracellularly recorded potentials from the postsynaptic giant axon. Prior to Cd^{2+} an EPSP/action potential complex was recorded (response with the shortest latency). Infusion of Cd^{2+} resulted in a decline in the EPSP so that threshold, the takeoff point of the action potential, was reached at progressively longer latencies. Transmission failed when the EPSP amplitude declined to below threshold (at 8 s after the onset of Cd^{2+} infusion). S = stimulus artifact; calibration: amplitude = 20 mV, time = 1 ms.

ligatures results in an effective perfusion of one (or both) of the ganglia *in vitro*. This could be demonstrated by simply perfusing dyes or fixative into the preparation.

Treatment with La^{3+} or Cd^{2+}, or removal of Ca^{2+} from the perfusion solution blocked synaptic transmission. The physiological bases for these findings are well established. The action potential in the presynaptic axon terminal triggers the voltage gated influx of Ca^{2+} (Katz and Miledi, 1965) which is believed to result in the fusion of transmitter filled vesicles with the nerve terminal membrane and the discharge of their contents into the synaptic cleft (*c.f.*, Heuser and Reese, 1973). The cations La^{3+} (Heuser and Miledi, 1971) and Cd^{2+} (Satoh *et al.*, 1982) are known to block Ca^{2+} entry into the nerve terminal (see Hagiwara and Byerly, 1981, for recent review). The effects of these two ions on the EPSP were similar, except that La^{3+} was irreversible, as in other preparations (Heuser and Miledi, 1971). Perfusion of La^{3+} or Cd^{2+} into the ganglion resulted in a decline in the EPSP with complete blockade of transmission in a minimum of 12 seconds. In fact, this latency does not take into consideration the time taken to clear the dead space in the cannula after switching from ASW to ASW plus the test ion, typically 3 to 4 seconds. Thus, arterial perfusion can block Ca^{2+} triggered transmitter release within 9 seconds of introduction, an access improvement of at least 100-fold.

Ca^{2+}-free medium had a similar effect to that of La^{3+} and Cd^{2+}. As reported previously removal of Ca^{2+} from the external medium eliminates the release of transmitter at the giant synapse (Miledi and Slater, 1966; Lester 1970). However, transmission is blocked at least 50 times faster with perfusion than by removing Ca^{2+} from the bathing medium, as reported in these previous studies. Re-introducing Ca^{2+} in ASW results in recovery of transmission, indicating that with the brief exposures necessary with the perfusion technique Ca^{2+}-free medium does not permanently damage the nerve terminal.

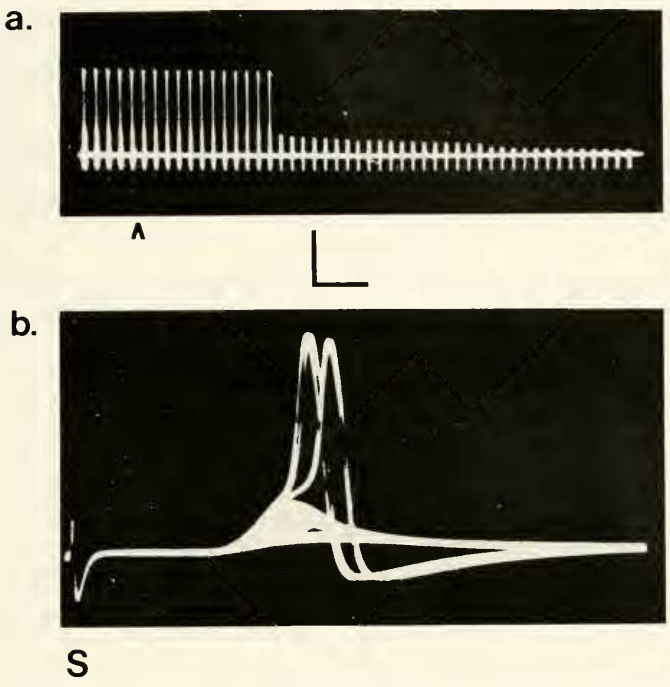

FIGURE 5. Effect of Ca^{2+}-free infusion on synaptic transmission at the giant synapse. a. Infusion of Ca^{2+}-free ASW (onset at arrow head) resulted in a failure of the action potential component of the intracellularly recorded evoked response in the postsynaptic giant axon followed by the gradual disappearance of the EPSP. Calibration: 50 mV, 5 s. b. Same preparation as in (a) at a fast trace sweep speed. The superimposition of EPSPs that are gradually declining amplitude obscures the visualization of individual traces. Calibration 20-mV, time = 1 ms.

Both La^{3+} and Cd^{2+} also had a limited depolarizing effect on the resting potential of the postsynaptic axon. The basis for this effect is not well established. Cd^{2+} has been reported to reversibly slow the activation kinetics of voltage sensitive K^+ channels (Gilley and Armstrong, 1982). If some of the K^+ channels were open at rest, their closure would result in a decline in the resting potential. Cd^{2+} and La^{3+} may also reduce the resting potential by direct effects on membrane ion permeability, for example La^{3+} increases the flux of Na^+ ions (P. DeWeer, pers. comm.).

The results of this study show that ion penetration into the stellate ganglion is greatly enhanced by arterial perfusion. Clearly substances can gain quick access to a *small fraction* of the giant synapse from the bathing medium; for example Ca^{2+} iontophoresed on to the presynaptic terminal in Ca^{2+}-free medium is capable of triggering limited transmitter release (Miledi and Slater, 1966). However, a substantial fraction of the presynaptic terminal must be inaccessible to bath application, as discussed above. The usefulness of the perfusion technique is that the ions must gain access to the entire synapse or it would not be possible to completely eliminate transmitter release. Similar arguments can be made for the postsynaptic giant axon where bath-applied glutamate fails to depolarize even after prolonged exposure at high concentrations (Kelly and Gage, 1969), whereas perfusion results in a depolarization that is detectable within seconds (Stanley, 1983, 1984).

Many other substances can also be introduced into the giant synapse by this

technique. Recently perfusion has been used to demonstrate and characterize a cholinergic receptor on the postsynaptic giant axon, and to compare the actions of a variety of glutaminergic and cholinergic agonists (Stanley, 1983, 1984). In addition, the perfusion technique is compatible with intracellular recording from the presynaptic giant axon (Stanley, 1984). The latter is perhaps the most exciting application for this technique: the examination of the effects of ions and pharmacological agents on the unique aspect of squid giant synapse, the accessible presynaptic giant axon terminal.

ACKNOWLEDGMENTS

The technical assistance of R. Mueller and secretarial help of C. F. Barlow-Salemi are gratefully appreciated. This study was supported by grants from NIMH 1 RO3 MH38359-01 and the Johns Hopkins Medical Institutions.

LITERATURE CITED

BRYANT, S. H. 1958. Transmission in squid giant synapses: the importance of oxygen and the effect of drugs. *J. Gen. Physiol.* **41**: 473–484.

BULLOCK, T. M., AND S. HAGIWARA. 1957. Intracellular recording from the giant synapse of the squid. *J. Gen. Physiol.* **40**: 565–577.

BULLOCK, T. H. 1948. Properties of a single synapse in the stellate ganglia of the squid. *J. Neurophysiol.* **42**: 609–616.

CHARTON, M. P., S. J. SMITH, AND R. S. ZUCKER. 1982. Role of presynaptic calcium ions and channels in synaptic facilitation and depression at the squid giant synapse. *J. Physiol.* **323**: 173–193.

ERULKAR, S. D., AND F. F. WEIGHT. 1977. Extracellular potassium and transmitter release at the giant synapse of squid. *J. Physiol. (Lond.)* **266**: 209–218.

GILLEY, W. F., AND C. M. ARMSTRONG. 1982. Divalent cations and the activation kinetics of potassium channels in squid axons. *J. Gen. Physiol.* **79**(2): 965–996.

HAGIWARA, S., AND L. BYERLY. 1981. Calcium channel. *Ann. Rev. Neurosci.* **4**: 69–125.

HEUSER, J. G., AND R. MILEDI. 1971. Effect of lanthanum ions on structure and function of frog neuromuscular junctions. *Proc. R. Soc. Lond. B* **179**: 247–260.

HEUSER, J. G., AND T. S. REESE. 1973. Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. *J. Cell. Biol.* **57**: 315–344.

KATZ, B., AND R. MILEDI. 1965. The effect of calcium on acetylcholine release from motor nerve terminal. *Proc. R. Soc. Lond. B* **161**: 196–503.

KELLY, J. S., AND P. W. GAGE. 1969. L-glutamate blockade of transmission at the giant synapse of the squid stellate ganglion. *J. Neurobiol.* **2**: 209–219.

KUSANO, K., D. R. LIVENGOOD, AND R. WERMAN. 1967. Correlation of transmitter release with membrane properties of the presynaptic fiber of the squid giant synapse. *J. Gen. Physiol.* **50**: 2579–2601.

LESTER, M. A. 1970. Transmitter release by presynaptic impulses in the squid stellate ganglion. *Nature* **227**: 493–496.

LLINAS, R. L., I. Z. STEINBERG, AND K. WALTON. 1981. Relationship between presynaptic calcium current and postsynaptic potential in squid giant synapse. *Biophys. J.* **33**: 323–352.

LLINAS, R., K. WALTON, AND V. BOHR. 1976. Synaptic transmission in squid giant synapse after potassium conductance blockade with external 3- and 4-aminopyridine. *Biophys. J.* **16**: 83–86.

MARTIN, R., AND R. MILEDI. 1975. A presynaptic complex in the giant synapse of the squid. *J. Neurocytol.* **4**: 121–129.

MILEDI, R. 1973. Transmitter release induced by injection of calcium ions into the nerve terminal. *Proc. R. Soc. Lond. B* **183**: 421–425.

MILEDI, R., AND SLATER. 1966. The action of calcium on the neuronal synapses in the squid. *J. Physiol.* 184, 473–498.

SATOH, E., A. FUMITOSHI, I. KATSUAKI, AND M. NISHIMURA. 1982. Mechanisms of cadmium induced blockade of neuromuscular transmission. *Eur. J. Pharmacol.* **77**: 251–257.

STANLEY, E. F. 1983. Depolarizing and desensitizing actions of glutaminagic and cholinergic agonists at the squid giant synapse. *Biol. Bull.* **165**: 533.

STANLEY, E. F. 1984. Action of cholinergic agents on the squid giant synapse. *J. Neurosci.* **4**: 1904–1911.

STANLEY, E. F., AND W. J. ADELMAN, JR. 1982. Perfusion of the squid stellate ganglion through its blood supply: Implications for morphological and physiological studies of the squid giant synapse. *Biol. Bull.* **163**: 403.

WEBB, G. D., W. D. DETTBARN, AND M. BRISIN. 1966. Biochemical and pharmacological aspects of the synapses of the squid stellate ganglia. *Biochem. Pharmacol.* **15**: 1813–1814.

WILLIAMS, L. W. 1909. *The Anatomy of the Common Squid.* Brill, Leiden, Holland. 92 pp.